Actinopolymorpha rutila sp. nov., isolated from a forest soil

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A Gram-positive, aerobic actinomycete, designated strain YIM 45725^T, was isolated from a forest soil sample in Yunnan Province, China. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate was a member of the family *Nocardioideae* and was related most closely to *Actinopolymorpha singaporensis* IM 7744^T (99.4 % 16S rRNA gene sequence similarity). Chemotaxonomic data, including cell-wall components, menaquinones and polar lipids, supported the affiliation of strain YIM 45725^T to the genus *Actinopolymorpha*. The results of physiological and biochemical investigations and DNA–DNA hybridization experiments demonstrated that strain YIM 45725^T differed from the single recognized species of the genus *Actinopolymorpha*. Strain YIM 45725^T is therefore considered to represent a novel species of the genus *Actinopolymorpha*, for which the name *Actinopolymorpha rutila* sp. nov. is proposed. The type strain is YIM 45725^T (=CCTCC AA 206004^T=DSM 18448^T).

The genus *Actinopolymorpha* was established by Wang *et al.* (2001) and it was placed within the family *Nocardioideae* (Nesterenko *et al.*, 1985). At the time of writing, the family *Nocardioideae* comprises the genera *Nocardioides* Prauser *et al.* 1976 (type genus), *Aeromicrobium* Miller *et al.* 1991, *Kribbella* Park *et al.* 1999, *Marmoricola* Urzì *et al.* 2000, *Actinopolymorpha* Wang *et al.* 2001 and *Jiangella* Song *et al.* 2005. During the course of research into unknown actinomycetes, an *Actinopolymorpha*-like strain (YIM 45725^T) was isolated from a forest soil collected in Yunnan Province, south-west China. In order to determine the taxonomic position of this organism, we investigated its morphological, physiological and biochemical characteristics and analysed its chemotaxonomic composition and 16S rRNA gene sequence. The results indicated that strain YIM 45725^T represents a novel species of the genus *Actinopolymorpha*.

Cultural characteristics of strain YIM 45725^T were determined by using 14-day-old cultures grown at 28 °C

on International *Streptomyces* Project (ISP; Shirling & Gottlieb, 1966) media 2, 3, 4 and 5, Czapek's agar plates (Pridham & Lyons, 1980), potato agar and nutrient agar. Colony colour was determined based on Kelly (1964). Morphological observations of cultures after incubation at 28 °C for 12 and 30 days on ISP 2 medium were made by light microscopy (BH-2; Olympus) and scanning electron microscopy (XL30; Philips).

Strain YIM 45725^T had morphological properties consistent with those of the genus *Actinopolymorpha*. The novel isolate formed branched hyphae of uneven thickness that fragmented into short chains or aggregates in the early stages of growth. The branched substrate mycelia later exhibited varied degrees of fragmentation, with short to elongated rod-like elements or V-shaped elements, and in the later stages of growth, poorly formed aerial hyphae were observed on ISP 2 medium. Spores or sporangia were not observed (see Supplementary Fig. S1 in IJSEM Online). Colonies developed on the tested media within 3–4 days were raised and varied from smooth to rugose. Strain YIM 45725^T grew well on ISP 2 and 5 media and potato agar and moderately well on ISP 3 and 5 media, Czapek's agar plates and nutrient agar. Strain YIM 45725^T developed pink orange–yellow to deep orange–yellow colony colours on all media tested (Table 1), but no diffusible pigments were produced.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 45725^T is EF601829.

Scanning electron and light micrographs of cells of strain YIM 45725^T and a phylogenetic tree based on a comparative 16S rRNA gene sequence analysis, showing the relationships between strain YIM 45725^T and related taxa, are available with the online version of this paper.

Table 1. Cultural characteristics of strain YIM 45725^T

Diffusible pigments were not produced on any of the media listed. ISP, International *Streptomyces* project.

Medium	Growth	Aerial mycelium	Substrate mycelium
Yeast extract-malt extract (ISP medium 2)	Good	Poor	Deep orange-yellow
Oatmeal agar (ISP medium 3)	Moderate	Absent	Brilliant orange-yellow
Inorganic salt-starch agar (ISP medium 4)	Moderate	Absent	Pink orange-yellow
Glycerol-asparagine (ISP medium 5)	Good	Absent	Brilliant orange-yellow
Czapek's agar	Moderate	Absent	Brilliant orange-yellow
Nutrient agar	Moderate	Absent	Pink orange-yellow
Potato agar	Good	Absent	Deep orange-yellow

Physiological characteristics, including sugar fermentation analyses, utilization of organic acids and carbohydrates and hydrolysis or decomposition of adenine, hypoxanthine, tyrosine, xanthine, casein, aesculin and urea, were evaluated as described by Gordon *et al.* (1974) after incubation at 28 °C for 15 days; the type strain of *Actinopolymorpha singaporensis* was also employed in these studies. Determination of enzyme activities was carried out by using the API ZYM test system (bioMérieux) according to the manufacturer's recommendations. Catalase activity was detected based on the production of bubbles after the addition of a drop of 3 % H₂O₂. Oxidase activity was determined based on the oxidation of 1 % *p*-aminodimethylaniline oxalate. Growth at different temperatures (4, 15, 20, 28, 37, 45 and 50 °C), NaCl concentrations (1, 3, 5, 7 and 10 %) and pH (4.0–10.0) was assessed after incubation at 28 °C for 7–15 days on ISP 2 medium.

Good growth occurred at 28 °C, but no growth occurred below 15 or above 37 °C. Growth was observed between pH 6 and 8 and at up to 5 % NaCl. Strain YIM 45725^T was oxidase- and catalase-positive. Differential physiological features between strain YIM 45725^T and *A. singaporensis* IM 7744^T are given in Table 2; other phenotypic characteristics of strain YIM 45725^T are given in the species description.

The amino acid and sugar patterns of the cell wall were determined as described by Stanek & Roberts (1974). Polar lipids were extracted as described by Minnikin *et al.* (1979) and identified by two-dimensional TLC and after spraying with specific reagents (Collins & Jones, 1980). Menaquinones were determined according to the procedures of Collins *et al.* (1977) and were analysed by HPLC as described by Tamaoka *et al.* (1983). Biomass for quantitative fatty acid analysis was prepared by scraping cells from TSB agar plates [trypticase soy broth (BBL), 3 % (w/v); Bacto agar (Difco), 1.5 % (w/v)] that had been incubated for 5 days at 28 °C. The fatty acids were extracted, methylated and analysed by using the standard MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The G+C content of the genomic DNA was determined by HPLC according to Mesbah *et al.* (1989).

Chemotaxonomically, strain YIM 45725^T contained chemical markers supporting its assignment to the genus *Actinopolymorpha*. The cell wall contained LL-diaminopimelic acid as well as ribose, glucose and galactose. Strain YIM 45725^T could be differentiated from the type strain of *A. singaporensis* based on the absence of rhamnose and presence of galactose. MK-9(H₄) was the predominant menaquinone. MK-9(H₆), MK-10(H₄) and MK-10(H₆) were also present. In contrast, the predominant menaquinone of *A. singaporensis* was MK-9(H₆). Phospholipids consisted of phosphatidylinositol mannosides, phosphatidylinositol, diphosphatidylglycerol and phosphatidylglycerol (type PI pattern *sensu* Lechevalier *et al.*, 1981). The major fatty acids (>10 %) in whole-cell methyl esters were iso-C_{16:0} (22.36 %), iso-C_{15:0} (22.09 %) and iso-C_{16:1} H (21.57 %). The DNA G+C content of strain YIM 45725^T was 67.7 mol%.

Extraction of genomic DNA and PCR amplification for 16S rRNA gene sequence analysis were performed as described by Li *et al.* (2007). The 16S rRNA gene sequence of strain YIM 45725^T was aligned with that of *A. singaporensis* IM 7744^T and of members of related genera retrieved from GenBank by using the CLUSTAL_X 1.8 program (Thompson *et al.*, 1997) based on BLAST results in the NCBI. Subsequently, phylogenetic analysis was performed by using the MEGA3 software package (Kumar *et al.*, 2004). Distances were calculated by using distance options according to the Kimura two-parameter model (Kimura, 1980, 1983) and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis (1000 resamplings) was used to evaluate the tree topology of the neighbour-joining data (Felsenstein, 1985).

To determine the phylogenetic position of strain YIM 45725^T, the almost-complete 16S rRNA gene sequence (1429 nt) was determined and subjected to comparative analysis. These comparisons showed clearly that strain YIM 45725^T was a member of the genus *Actinopolymorpha* within the family *Nocardioidaceae*. Strain YIM 45725^T showed the highest 16S rRNA gene sequence similarity to *A. singaporensis* IM 7744^T (99.4 %). Significantly lower levels of similarity (<93.0 %) were found to all other taxa investigated. The neighbour-joining phylogenetic tree is

Table 2. Differential characteristics between strain YIM 45725^T and its closest phylogenetic neighbour, *Actinopolymorpha singaporensis* IM 7744^T

Both strains are positive for the hydrolysis of casein, hypoxanthine, xanthine, glycine, L-proline, Tween 20 and gelatin, but are negative in tests for the degradation of Tween 80, adenine, cellulose and starch, and for the utilization of D-glucose, D-fructose, maltose, dextrin, salicin, malonate, L-rhamnose, D-xylose, D-mannitol, sucrose, *myo*-inositol and glycerol as carbon sources. Both are positive for acid production from D-fructose, sucrose, glucose and D-xylose. +, Positive; –, negative.

Characteristic	YIM 45725 ^T	<i>A. singaporensis</i> IM 7744 ^T
Utilization of substrate as carbon source		
Raffinose	+	–
Sorbitol	–	+
Lactose	+	–
Cellobiose	+	–
D-Arabinose	+	–
Sodium citrate	–	+
Sodium succinate	+	–
Acid production from:		
Mannose	+	–
Maltose	+	–
L-Rhamnose	–	+
Sorbitol	–	+
<i>myo</i> -Inositol	–	+
D-Galactose	–	+
Milk coagulation	–	+
Milk peptonization	–	+
Reduction of nitrate	–	+
Growth temperature range (°C)	15–37	25–37

available as Supplementary Fig. S2 in IJSEM Online. It is evident from this tree that strain YIM 45725^T forms a distinct cluster with *A. singaporensis* IM 7744^T in the family *Nocardioidaceae*.

Given the high level of 16S rRNA gene sequence similarity between strain YIM 45725^T and *A. singaporensis* IM 7744^T, DNA–DNA hybridization studies were performed according to the methods described by He *et al.* (2005) to confirm whether strain YIM 45725^T represented a distinct species. Strain YIM 45725^T displayed a relatively low level of DNA–DNA relatedness (mean of 33.2 %) to *A. singaporensis* KCTC 19907^T (=IM 7744^T), a result that is below the cut-off point recommended by Wayne *et al.* (1987) for circumscription of bacterial genomic species, and confirmed the separation of strain YIM 45725^T from its nearest phylogenetic neighbour. This was also supported based on some differential phenotypic characteristics between the two taxa, which are detailed in Table 2.

The genotypic and phenotypic data presented indicate that strain YIM 45725^T represents a novel species of the genus *Actinopolymorpha*, for which the name *Actinopolymorpha rutila* sp. nov. is proposed.

Description of *Actinopolymorpha rutila* sp. nov.

Actinopolymorpha rutila (ru.ti'la. L. fem. adj. *rutila* red inclining to golden–yellow, referring to the colour of colonies produced).

Grows at 15–37 °C (optimum 28 °C), pH 6–8 and in the presence of up to 5 % NaCl on ISP 2 medium. Colonies develop well within 3–4 days and vary from smooth to rugose; colony colour is dependent on the medium used for growth, varying from pink orange–yellow to deep orange–yellow. Diffusible pigments are not formed. Forms branched substrate mycelia of uneven thickness that fragment into short chains or aggregates in the early stages of growth; branched hyphae later exhibit varied degrees of fragmentation with short to elongated rod-like elements or V-shaped elements; in the later stages of growth, poorly formed aerial hyphae are seen on ISP 2 agar. Spores and sporangia are absent. Gram-positive. Catalase- and oxidase-positive. H₂S is not produced. In addition to the properties shown in Table 2, assimilates ribose, mannose, melibiose, maltose, melezitose, dextrin, xylitol, adonitol, D-galactose, salicin and malonate as sole carbon sources, but not dulcitol, arabinol, acetate, citrate or oxalate. Hydrolyses hypoxanthine, xanthine, casein, L-alanine, L-asparagine, L-arginine, glycine, L-histidine, L-hydroxyproline, phenylalanine, L-proline, L-cysteine, L-valine, urea and Tween 20, but not L-tyrosine, adenine, glucosamine, Tween 60, Tween 80, cellulose or DNA. Acid is produced from D-fructose, ribose, fucose, raffinose, sucrose, erythritol, glucose and D-xylose. Positive for the enzymes alkaline phosphatase, esterase (C4), esterase lipase (C8), α -glucosidase, β -glucosidase, α -galactosidase, β -galactosidase and α -mannosidase, but negative for *N*-acetyl- β -glucosaminidase, lipase (C14), β -glucuronidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, leucine arylamidase, cystine arylamidase, valine arylamidase, α -fucosidase, chymotrypsin and trypsin. Tests positive for gelatin, but negative for nitrate reduction, starch hydrolysis, milk coagulation and peptonization. The diagnostic amino acid of the peptidoglycan is LL-diaminopimelic acid. Cell-wall hydrolysates contain ribose, glucose and galactose. MK-9(H₄) is the predominant menaquinone; MK-9(H₆), MK-10(H₄) and MK-10(H₆) are also present. Phospholipids consist of phosphatidyl-inositol mannosides, phosphatidyl-inositol, diphosphatidyl-glycerol and phosphatidyl-glycerol. The major cellular fatty acids (>1 %) are iso-C_{16:0} (22.36 %), iso-C_{15:0} (22.09 %), iso-C_{16:1} H (21.57 %), iso-C_{17:0} (8.92 %), anteiso-C_{17:0} (8.30 %), iso-C_{14:0} 3-OH (3.80 %), anteiso-C_{15:0} (3.66 %), iso-C_{14:0} (2.59 %), iso-C_{18:1} H (1.64 %), C_{15:0} 3-OH (1.48 %) and 10-methyl C_{17:0} (1.06 %). The DNA G + C content of the type strain is 67.7 mol%.

The type strain, YIM 45725^T (=CCTCC AA 206004^T=DSM 18448^T), was isolated from a soil sample collected in Yunnan Province, south-west China.

Acknowledgements

This research was supported by the National Basic Research Program of China (No. 2004CB719601), the National Natural Science Foundation of China (Nos. 30560001, 30600001), the Yunnan

Provincial International Cooperative Program (No. 2005GH21) and the Ministry of Science and Technology, PR China (2006DFA33550). W. J. L. was also supported by the Program for New Century Excellent Talent in University (NCET).

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